

Effect of Acute Elevation of Plasma Glycerol, Triglyceride and FFA Levels on Glucose Utilization and Plasma Insulin

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SUMMARY

The effects of raised plasma levels of glycerol, free fatty acids (FFA) and triglyceride on the disappearance rate (K_g) of intravenously administered glucose (25 gm.) and on the response of plasma immunoreactive insulin (IRI) to intravenous glucose were studied in thirty-six subjects. Increase of plasma glycerol level by oral glycerol augmented the K_g but not the glucose-stimulated plasma insulin level. Alimentary hyperglyceridemia enhanced the disappearance rate of glucose and the response of plasma insulin to glucose. Heparin injected intravenously during alimentary glyceridemia caused a five- to seven-fold increment of the levels of plasma FFA and glycerol but did not influence either the disappearance rate of glucose or the response of plasma insulin to glucose. Heparin injected in fasting state slowed significantly the disappearance rate of glucose, but did not change the insulin response. *DIABETES* 17:76-82, February, 1968.

According to the "glucose-fatty acid cycle" concept,¹ disturbances in glucose tolerance and insulin sensitivity are secondary phenomena to an augmented fatty acid release from adipose tissue. In this hypothesis diabetes mellitus begins with elevated levels of plasma free fatty acids (FFA) which then is followed by impaired utilization of glucose and hyperinsulinemia.

In support of this theory a number of *in vitro* experiments have revealed an inhibitory effect of fatty acids on the oxidation of glucose in muscular tissue.²⁻⁵ Moreover, an inverse correlation between plasma FFA levels and glucose turnover rate has been demonstrated in the dog.⁶ On the other hand, experimental evidence that an increase of plasma FFA levels actually decreases glucose tolerance or that a decrease of an elevated level of plasma FFA increases glucose tolerance in man is so

far insufficient. Impaired glucose tolerance caused by norepinephrine infusion can be corrected if the FFA elevation is prevented by nicotinic acid,⁷ but reduction of the plasma FFA level by nicotinic acid in obese subjects does not improve the utilization of glucose.⁸ The profound hemodynamic changes occurring in these experiments may have influenced the results, however. Hyperglyceridemia and increased plasma FFA levels produced by intravenous infusion of lipid emulsion have been found to decrease glucose tolerance⁹⁻¹¹ in spite of an augmentation of plasma insulin response to glucose.^{9,10} On the contrary, no effects from an oral fat load were observed.¹² Heparin administered to fasting subjects caused a manyfold increment of plasma FFA content but did not appear to influence glucose tolerance or the response of plasma insulin to glucose.¹² Schalch and Kipnis observed, however, a significant decrease in the disappearance rate of glucose when heparin was administered after a fat meal.¹² This finding was not confirmed by Balasse.¹³

As all of these experimental designs have simultaneously modified the plasma levels of FFA, glycerol, and triglycerides it is difficult to draw any conclusions on the possible interference of each of these plasma constituents with glucose metabolism. It was therefore decided to study glucose tolerance and plasma insulin in relation to acute changes of plasma triglyceride, glycerol and FFA levels each induced separately.

MATERIAL AND METHODS

Experimental subjects

Experiments were conducted in thirty-six subjects, four of whom were healthy medical students and the others patients admitted to the hospital because of minor nonmetabolic illnesses. All had normal serum triglyceride (< 150 mg. per 100 ml.) and cholesterol (< 300 mg. per 100 ml.) levels. The fasting blood

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glucose levels were normal, but in some the K-values of the intravenous glucose tests were subnormal. The ages of the subjects ranged from twenty-five to forty-five years; the mean was thirty-eight years. During the tests the patients were on an institutional hospital daily diet equal to about 2,000 calories of which two fifths were derived from carbohydrates. The medical students continued their own diet which was somewhat higher in calories.

Experimental design

The following four arrangements (A-D) were employed. In all experiments each subject served as his own control. To exclude the possibility that the pro-

cedure as such could modify the results, the order of the different tests varied from one subject to another.

A. The effects of elevated plasma glycerol on glucose utilization and glucose-stimulated insulin were measured as the differences between intravenous glucose tolerance tests (IGT) carried out in each subject on consecutive days with and without preceding oral glycerol load (10 gm. or 109 mmoles as a 10 per cent aqueous solution: glycerol Bidest "Merck") administered fifteen minutes before glucose injection (figure 1A).

B. The effects of alimentary triglyceridemia were studied by comparing an intravenous glucose tolerance test carried out after twelve hours' fast to that per-

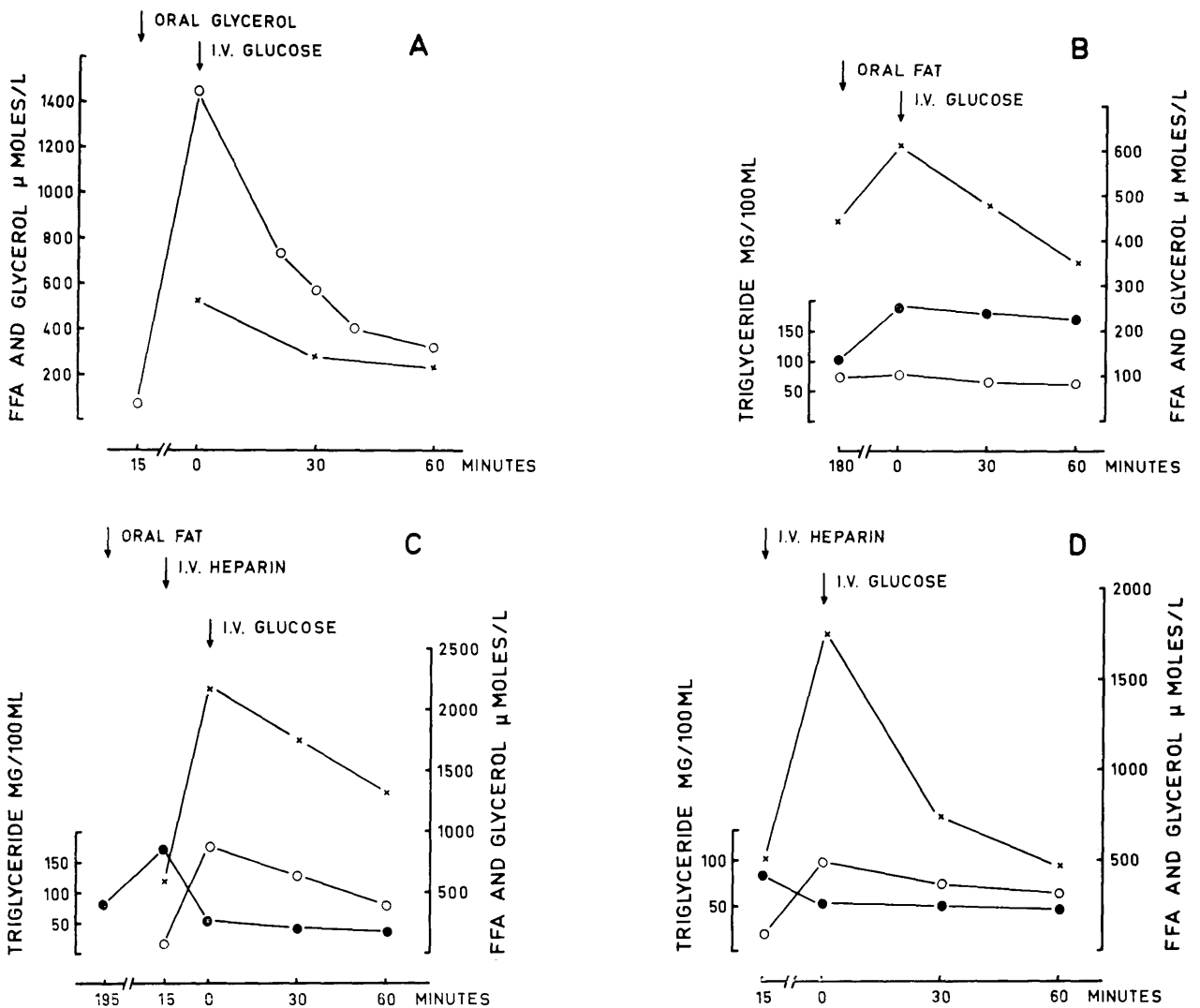


FIG. 1. The mean levels of plasma FFA (x), glycerol (O) and triglycerides (●) in different experimental conditions: A. Effect of oral glycerol (fourteen subjects). B. Effect of oral fat (eighteen subjects). C. Effect of heparin during alimentary hyperglyceridemia (eleven subjects). D. Effect of heparin (eight subjects).

formed three hours after an oral fat load. Sixty grams of butter fat as 40 per cent cream were ingested at 6 a.m. after a nine-hour fast and the glucose was injected three hours later at the estimated peak of alimentary hyperlipemia (figure 1B).

C. To study the influence of elevated FFA level combined with moderate glycerolemia on an intravenous glucose tolerance test, and on plasma insulin the subjects were first given a fat meal as in design B. But 50 mg. of heparin were injected intravenously fifteen minutes before the start of glucose injection (figure 1C).

D. In this group IGT was carried out fifteen minutes after injection of 50 mg. heparin. The test was preceded by a twelve hours' fasting period (figure 1D).

Intravenous glucose tolerance test

An intravenous injection of 25 gm. of glucose was given as a 50 per cent solution during two minutes.

Samples of venous blood were drawn at ten-minute intervals during fifty minutes. The disappearance rate was expressed as the K-value (percentage decline per minute) obtained from the slope of total blood glucose (basal plus load) curve on a semi-logarithmic scale. Assay of plasma insulin was made before and at 5, 15, 30, 45 and 60 minutes after the injection of glucose.

Analytical procedures

Plasma FFA was determined according to the method of Trout et al.¹⁴ and plasma triglycerides by the method of Carlson.¹⁵ Plasma glycerol was measured as described by Wieland¹⁶ without precipitation of proteins. The o-toluidine method¹⁷ was employed for the blood glucose determination from venous blood. Plasma insulin was assayed by immunoprecipitation,¹⁸ as presented in detail previously by Nikkilä et al.¹⁹ Sodium EDTA was used as an anticoagulant, and all determinations were made in duplicate.

TABLE 1

Individual values of plasma immunoreactive insulin (response to intravenous glucose) and values indicating the disappearance rate of glucose (K-value) in different experimental conditions. The table includes only those subjects in whom plasma insulin has been determined.

Subject No.	K-value per cent minutes	IVG IN FASTING STATE									
		Immunoreactive insulin (micro U./ml.)									
		Minutes									
		0	5	10	15	20	30	40	45	50	60
1	2.12	19	75	—	71	—	39	—	35	—	25
2	1.41	12	25	—	32	—	33	—	29	—	21
3	—	6	35	—	36	—	35	—	13	—	25
4	2.66	16	82	—	95	—	70	—	32	—	17
5	2.31	10	50	—	37	—	25	—	—	—	28
6	1.54	10	—	—	24	—	18	—	15	—	15
7	—	4	20	—	17	—	45	—	12	—	8
8	1.12	4	23	—	19	—	25	—	19	—	6
9	1.12	8	—	—	43	—	36	—	40	—	24
10	1.11	1	19	—	16	—	19	—	25	—	16
11	0.53	9	18	—	7	—	9	—	5	—	5
12	2.20	24	—	—	64	—	62	—	42	—	29
13	2.16	6	40	—	19	—	14	—	11	—	7
14	2.58	13	30	—	16	—	16	—	13	—	12
15	1.54	17	40	—	33	—	23	—	19	—	17
16	1.03	19	—	—	109	—	78	—	80	—	73
17	1.14	12	—	—	41	—	66	—	37	—	31
18	1.26	12	79	48	—	57	38	33	—	35	30
19	1.22	15	65	59	—	38	38	27	—	—	22
20	1.38	7	18	12	—	13	13	16	—	15	3
21	2.35	8	150	100	—	66	68	34	—	23	15
22	0.99	17	49	45	—	38	38	35	—	28	34
23	1.11	21	54	46	—	38	38	37	—	41	34
24	1.16	20	20	42	—	53	38	38	—	40	37
25	1.03	28	70	90	—	48	50	19	—	21	43
Mean	1.48	12.7	49.6	51.5	39.4	43.8	37.3	29.8	26.6	29.0	23.0
		IVG AFTER ORAL GLYCEROL									
1	2.66	23	127	—	99	—	51	—	22	—	7
2	1.73	17	29	—	31	—	30	—	24	—	20
3	—	6	26	—	25	—	27	—	20	—	11
4	3.30	16	59	—	62	—	43	—	13	—	13
5	2.72	14	82	—	50	—	37	—	—	—	13
12	2.30	20	—	—	76	—	49	—	33	—	23
13	2.23	6	48	—	20	—	18	—	12	—	8
14	1.58	5	24	—	15	—	15	—	14	—	14

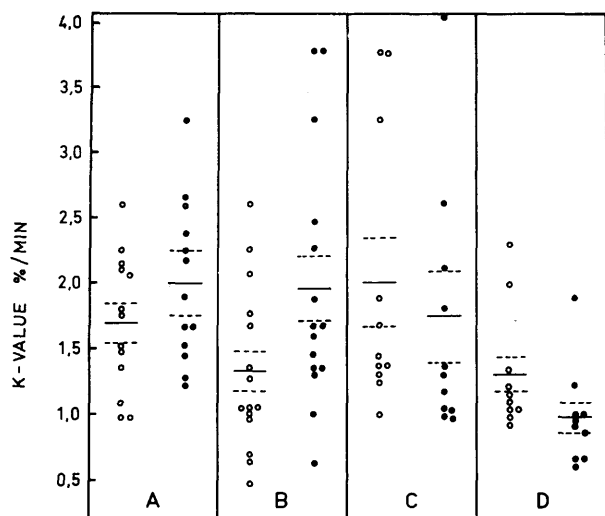


FIG. 2. Disappearance rate of intravenous glucose (25 gm.) in different experimental conditions. Solid line = the mean values, dotted lines = standard errors of the mean.

A. Open dots = intravenous glucose test. Black dots = intravenous glucose test after ingestion of glycerol (10 gm.).

B. Open dots = intravenous glucose test. Black dots = intravenous glucose test after ingestion of fat (60 gm.).

C. Open dots = intravenous glucose test after ingestion of fat (60 gm.). Black dots = intravenous glucose test after ingestion of fat + intravenous injection of heparin (50 mg.).

D. Open dots = intravenous glucose test. Black dots = intravenous glucose test after intravenous injection of heparin (50 mg.).

The actual rise in plasma insulin from the fasting state was used in determining insulin response during

the test conditions. The subjects served as their own controls, and the mean difference in response was com-

TABLE 1 (continued)

Individual values of plasma immunoreactive insulin (response to intravenous glucose) and values indicating the disappearance rate of glucose (K-value) in different experimental conditions. The table includes only those subjects in whom plasma insulin has been determined.

Subject No.	K-value per cent minutes	IVG AFTER ORAL GLYCEROL									
		Immunoreactive insulin (micro U./ml.)									
		Minutes									
		0	5	10	15	20	30	40	45	50	60
15	1.73	13	35	—	43	—	35	—	30	—	19
16	1.32	20	—	—	124	—	138	—	117	—	85
17	1.51	14	—	—	53	—	32	—	33	—	32
Mean	2.10	14.0	53.7	—	54.3	—	43.1	—	31.8	—	22.2
		IVG AFTER ORAL FAT									
1	3.85	28	102	—	78	—	35	—	25	—	23
2	1.41	19	49	—	37	—	36	—	35	—	24
3	1.28	18	63	—	60	—	64	—	63	—	47
4	3.85	19	135	—	94	—	41	—	15	—	15
5	3.30	10	72	—	47	—	42	—	—	—	10
6	—	11	19	—	14	—	14	—	12	—	7
7	2.77	2	18	—	13	—	11	—	7	—	4
8	1.73	7	44	—	32	—	24	—	24	—	13
9	1.65	1	36	—	36	—	24	—	32	—	25
10	2.52	16	67	—	53	—	43	—	40	—	30
11	0.67	8	26	—	11	—	13	—	10	—	10
Mean	2.30	12.6	57.3	—	43.1	—	31.5	—	26.3	—	18.9
		IVG AFTER ORAL FAT AND IV HEPARIN									
1	2.66	33	110	—	91	—	39	—	36	—	29
2	1.23	9	20	—	21	—	35	—	30	—	18
3	2.16	5	50	—	46	—	43	—	37	—	13
4	4.95	7	98	—	72	—	22	—	15	—	11
5	1.85	15	37	—	44	—	39	—	—	—	18
Mean	2.57	13.8	62.4	—	54.8	—	35.6	—	29.5	—	17.8
		IVG AFTER IV HEPARIN									
18	1.07	19	86	53	—	42	35	38	—	42	37
19	0.73	18	30	33	—	51	51	39	—	43	39
20	0.96	11	45	17	—	22	19	23	—	17	18
21	1.95	8	190	49	—	37	28	33	—	17	20
22	0.69	17	44	38	—	41	35	39	—	—	40
23	0.73	29	47	43	—	44	40	45	—	46	43
24	1.07	23	66	50	—	45	41	41	—	33	38
25	0.99	12	86	70	—	45	39	49	—	38	43
Mean	1.39	17.1	74.2	44.1	—	40.8	36.0	38.3	—	33.7	34.7

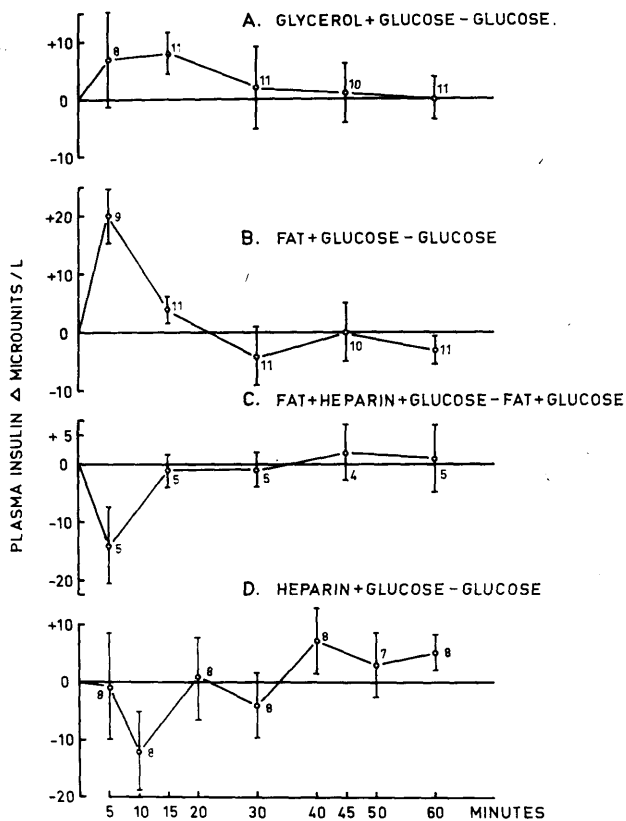


FIG. 3. Response of plasma immunoreactive insulin (IRI) to intravenous glucose (25 gm.) in different experimental conditions. The dots indicate the mean differences of plasma IRI values measured in two separate procedures. The upright lines give the standard errors of the mean and the figures to the number of subjects at each timepoint.

A. Effect of ingestion of glycerol (10 gm.) on IRI response to glucose as compared to glucose without glycerol.

B. Effect of ingestion of 60 gm. of fat (40 per cent cows' cream) on IRI response to glucose as compared to glucose without fat.

C. Effect of ingestion of 60 gm. of fat + intravenous heparin (50 mg.) on IRI response to glucose as compared without heparin.

D. Effect of intravenous heparin (50 mg.) on IRI response to glucose as compared to glucose without heparin.

pared to no difference by the *t* test. Changes in glucose disappearance rates were treated in similar fashion.

RESULTS

Effect of glycerol. Ten grams of glycerol fed to fourteen subjects caused within fifteen minutes an average thirtyfold increment of the plasma glycerol content (figure 1A). In thirteen subjects the mean disappearance rate (K-value) after glycerol was 2.06 ± 0.25 in contrast to 1.75 ± 0.14 in the control state

($p < 0.01$). The data are shown in figure 2A. In eleven subjects the response of plasma insulin to glucose was determined. Two of these subjects had abnormally low K-values (table 1). Glycerol did not increase significantly the response of plasma insulin to glucose (figure 3A and table 1).

Effect of alimentary hyperglyceridemia. Of eighteen subjects studied, eight had a normal (> 1.2) and the other eight subnormal (< 1.2) K-value, in two subjects the exact K could not be calculated (table 1). The fat load caused in three hours an average twofold increment of plasma triglyceride content and a slight elevation of plasma FFA, whereas plasma glycerol levels remained unchanged (figure 1B). The fat load improved the glucose utilization as indicated by an increase of K from the control mean value of 1.38 ± 0.15 to 2.01 ± 0.25 ($p < 0.01$) during alimentary lipemia. The improvement was found also in the cases with subnormal K-values.

The response of plasma insulin to intravenous glucose with and without preceding fat meal was studied in eleven subjects (table 1). The fasting insulin level remained unchanged after the fat load, but comparison of the insulin curves after intravenous glucose in these two tests revealed that fat ingestion caused a significantly higher insulin response at five ($p < 0.001$) minutes (figure 3B). No differences were apparent in the insulin response between the subjects with normal and abnormal K-values.

Effect of heparin during alimentary hyperglyceridemia. This test was carried out in eleven subjects (four cases with K-value < 1.2). Heparin injected during alimentary lipemia caused a rapid five to sevenfold increment in plasma FFA and glycerol levels with a simultaneous fall of plasma triglyceride content (figure 1C). The K-value did not differ from that obtained by the fat load only (figure 2C). Also, the plasma insulin response to IGT remained unchanged. The mean K-values and insulin responses were of the same magnitude as those obtained in the intravenous glucose tolerance tests made after a twelve hours' fast without any preceding treatment.

Effect of heparin without fat meal. Heparin was injected without a preceding fat meal in eight subjects (four normal, four abnormally low K-values). There was a three- to fivefold increase of plasma FFA and glycerol levels and a significant fall of plasma triglyceride (figure 1D). The K-value was lower in each case, the average (figure 2D) being 1.36 ± 0.13 before and 1.03 ± 0.11 after the heparin ($p < 0.001$). The

response of plasma IRI was not significantly influenced by heparin (table 1, figure 3D).

DISCUSSION

High concentrations of glycerol augmented the rate of glucose disposal, the increase possibly being due to stimulation of insulin secretion. It is not known whether glycerol has a direct stimulating effect on β -cells or if it could stimulate insulin secretion indirectly through conversion to glucose either in β -cells or in liver. Since ingestion of glycerol raises the fasting level of plasma IRI only inconsistently,²⁰ however, it is uncertain that glycerol per se stimulates the secretion of endogenous insulin. As the release of insulin can be stimulated by very small increment of blood glucose²¹ it is possible that glycerol could influence insulin secretion by a slight hyperglycemic effect.

In the present study fat ingestion increased glucose-induced insulin secretion and, correspondingly, the glucose disposal rate. This result is different from that of Schalch and Kipnis,¹² who could not find any effect of corn oil. Their conclusion is based only on three determinations, however, and on the use of area measurement for total insulin secretion. The latter method is open to some criticism because the area under the plasma insulin curve may be identical in two cases, one with high insulin secretion and rapid glucose disposal and another with low insulin secretion response and slow insulin and glucose decay rates. The mechanism by which fat could facilitate the insulin release is not understood. Although administered fat emulsion may influence the response of plasma insulin to glucose,⁹⁻¹¹ insulin-stimulating substances released from the intestinal wall²²⁻²⁵ should be considered as possible mechanisms for increased insulin secretion by fat meal. On the other hand, studies in progress indicate that of medium chain triglycerides (MCT), corn oil and cocoa butter, only MCT improves the glucose disappearance rate (K-value). Therefore, short chain fatty acids that are present in butterfat and are transported through portal blood directly from gut to the liver may be responsible for the improved disappearance rate caused by butterfat loading.

It cannot be stated that the present demonstration of increased insulin secretion during alimentary lipemia accounts for the hyperinsulinemia found in cases of endogenous hyperglyceridemia.²⁶⁻²⁹ Such a conclusion would be premature for several reasons. The plasma insulin response is highly variable in hyperglyceridemic patients,³⁰ the changes in glucose and/or insulin homeostasis may be primary pathogenetic events, and

sustained hyperglyceridemia may be associated with metabolic alterations very different from those occurring during acute induction of hyperglyceridemia.

The disappearance rate of glucose during alimentary glyceridemia was not influenced by heparin, whereas in fasting state heparin appeared to lower it significantly. In both experimental conditions the curves showing the effect of the administration of heparin on the levels of plasma insulin were very similar: There was a trend to a reduced response of insulin to glucose.

The results appear to conflict with those obtained by Schalch and Kipnis,¹² for these workers were not able to show that heparin influenced either the disappearance rate of glucose or the response of plasma insulin to glucose. In addition, Balasse¹³ did not observe any effect on the disposal rate of glucose of heparin alone or combined with fat. On the other hand, Schalch and Kipnis¹² did show that heparin administered during alimentary glyceridemia slowed the disappearance rate of glucose in spite of higher response of plasma IRI. Although they did not take into account the possible effect of the fat load as such, there remains a difference between their results and ours since in the present study the K-value and insulin response were not influenced by heparin given during alimentary lipemia.

It is not clear why heparin caused a decrease in the mean K-value without change in insulin levels. The results may have been due to (1) inhibition of insulin secretion either by heparin per se or by the heparin-induced increase of the levels of plasma FFA, (2) inhibition of uptake of glucose by tissues without any effect of secretion of insulin from beta cells. The possibility exists that the hyperglycerolemia masked the eventual inhibiting effect of FFA.

According to the concept of Randle et al.¹ free fatty acids have an inhibitory effect on the utilization of glucose by muscle tissue whereas fatty acids may even enhance the uptake of glucose by adipose tissue.³¹ Fatty acids perfused to the liver increase the output of glucose,³² but whether the fatty acids interfere with hepatic glucose utilization is not known. The net effect of all these metabolic influences on glucose disposal rate is unpredictable, and may depend on the relative amounts of muscle and adipose tissue as well as on the state of feeding. This possibly could explain the variable results obtained by different investigators.

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